IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Cantor et al.	Confirmation No.:	6905
Application No.:	10/655,762	Group No.:	1637
Filed:	September 5, 2003	Examiner: KIM, YOUNG J	
		Customer No.:	

For: QUANTIFICATION OF GENE EXPRESSION

DECLARATION OF DR. CHUNMING DING

I, Chunming Ding, M.S., Ph.D., declare as follows:

1. I am a co-inventor in the above-identified patent application.

2. A true copy of my current *curriculum vitae* is attached herewith.

3. I am aware that the Examiner has cited the following two articles in connection with the examination of the above-identified patent application: Becker-André and Hahlbrock, Nucleic Acid Research 17(22)9437-9446, 1998 ("Becker") and Amexis et al., Proc. Natl. Acad. Sci. U.S.A. 98(21):12097-12102, 2001 ("Amexis").

4. I am aware that the Examiner has argued that "[o]ne of ordinary skill in the art at the time the invention was made would have had a reasonable expectation of success at combining the teachings [of Becker and Amexis] since methods of quantification employing mass spectrometry, such as SNuPE (single nucleotide primer extension), have been well established." Page 7, first par. of March 8, 2007 Office Action. Emphasis added.

5. I disagree for the reasons explained in detail below.

6. Both of the cited articled describe looking at a single target in a uniplex reaction as explained in further detail below.

7. Amexis tested the MassArray system for relative quantification of two allelic virus variants of the same nucleic acid sequence that differed from each other in one reaction. See,

e.g., page 12100, first col., last paragraph. They did not use a standard and thus the measurement was a relative quantification of the amount of one variant against the other.

8. As described in Table 1, lower part, Amexis used only one extension primer in each reaction. Therefore, the maximum number of extension products in each reaction was two if both alleles were present. The authors did not combine the analysis of any different polymorphic markers.

9. Becker quantified a nucleic acid using a standard that differed by one nucleotide so that a restriction enzyme would digest one of the amplified nucleic acids.

10. Becker analyzed the amount of only one target nucleic acid, i.e., one nucleic acid molecule, per reaction.

11. The fact that Amexis describes overcoming the problems in the quantification reactions using enzymatic reactions by the use of MassArray technology with a primer extension reaction (see, e.g., page 12101, second column, last paragraph), does not in any way teach or suggest that the method can be used to look at multiple targets in a sample in a single reaction, i.e., using a multiplex method. As described above, Becker does not teach multiplexing either.

12. Accordingly, both articled cited by the Examiner describe a uniplex system of nucleic acid quantification. To my knowledge, prior to our discovery that multiplexing indeed is possible using the mass spectrometric detection system, no one had performed mass spectrometric analysis using multiplex detection, certainly not for the purpose of absolute quantification of nucleic acids.

13. The MassArray detection system is a very sensitive detection method. Therefore, this system was thought to be very sensitive also to artifacts in detection mixtures.

14. In the present application, we tested, and surprisingly found that we were able to look at and accurately quantify, a plurality of nucleic acid sequences, i.e., more than two targets. We also found that we can accurately quantify multiple target sequences in the same reaction. We tested gene expression quantification for multiple targets with a triplex PCR of three groups of sequences IL6, mcl1 and glut3 that were all co-amplified with their respective standards in the same reaction for PCR and primer extension. Thus, the reaction mixture contained six different

nucleic acids (3 sets of sequences with their respective standards). We found, that the extension products were clearly separated in the mass spectrum and quantified by their peak areas (Exhibit A). It is evident from the mass spectrum that high level of multiplexing can be achieved because our triplexing led to no deterioration in the precision of the method. This permits one to further increase the throughput and reduce cost for large scale gene expression analysis.

15. Exhibit A shows one of our multiplexing real competitive PCR (rcPCR) assays which was performed prior to the filing of the current application. Genes IL6, mcl1 and glut3 and their respective standards were co-amplified in the same reaction and the mass spectrum is shown here. P following the gene name stands for not extended extension primer. S stands for the extended oligo from the standard DNA. C, G and A stand for the specific alleles of the three genes containing a C, G, or A base at the mutation site. The quantification results by multiplex reactions agree well with those from uniplex reactions.

16. We have shown that the method can be used to quantify at least about 20 targets in one multiplex reaction.

17. In paragraph [008] of the specification, we teach that a plurality of target nucleic acids can be determined in a biological specimen. See also [0035].

18. In summary, multiplex quantification, particularly using an extremely sensitive detection method such as mass spectrometric analysis, was not something scientists did or considered prior to our discovery. Typically, one would have expected to run into severe problems because of background noise if multiple peaks were to be analyzed in the same reaction.

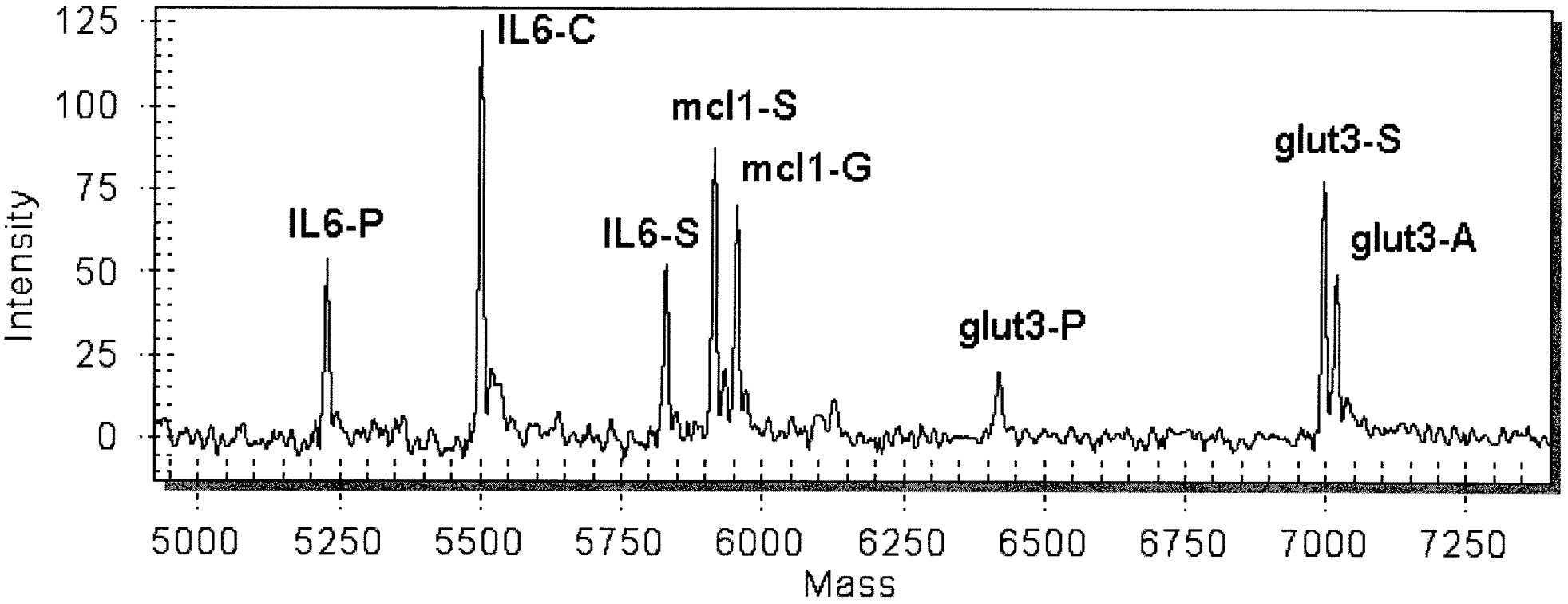
19. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information are believed to be true, and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, and that such willful false statements may jeopardize the validity of the application or any patent that issues therefrom.

Sept 21, 2007

Date

Chunming Ding

EXHIBIT A


125
100
75
50
25
0
Intensity
IL6-C
mcl1-S
mcl1-G
glut3-S
IL6-P
IL6-S
glut3-A
glut3-P
5000
5250
5500
5750
6000
6250
6500
6750
7000
7250
Mass

CURRICULUM VITAE

DING, CHUNMING

M.S., Ph.D.

PERSONAL INFORMATION

NAME:	Ding, Chunming
DATE OF BIRTH:	22nd June, 1975
PLACE OF BIRTH:	Shanghai, China
ADDRESS:	Centre for Emerging Infectious Diseases Faculty of Medicine The Chinese University of Hong Kong Prince of Wales Hospital Shatin, New Territories Hong Kong
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PRESENT APPOINTMENT

Assistant Professor — Jan, 2005-present
Faculty of Medicine,
The Chinese University of Hong Kong

EDUCATION

Bachelor of Science (Fudan University)	1993-1997
Master of Science (Brandeis University)	1997-2000
Doctor of Philosophy (Boston University)	2000-2003

PREVIOUS APPOINTMENTS

Research Assistant Professor — 2003-2005
Center for Advanced Biotechnology
and Bioinformatics Program
Boston University

RESEARCH INTERESTS

Molecular diagnostics using cell-free nucleic acids in body fluids
Viral mutations in response to antiviral treatment

Bacteria biota in human digestive tract
SNP genotyping and haplotyping
Gene expression and alternative splicing

SCHOLARSHIPS AND PRIZES

- First Prize, National Olympic Chemistry Contest, China 1993
- Runner up award for short talks in The 3rd International Symposium on Current Technologies for Gene Expression Analysis, Vanderbilt, USA 2002
- Predoctoral Travel Award, American Society for Biochemistry and Molecular Biology San Diego, USA 2003

MEMBERSHIP OF LEARNED SOCIETIES

- American Society for Biochemistry and Molecular Biology
- American Society of Human Genetics

INTRAMURAL SERVICE

- Member, Working Group on Research, Stanley Ho Centre for Emerging Infectious Diseases, 2005-present
- Member, Research and Education Committee, School of Public Health, Chinese University of Hong Kong, 2006- present

SERVICE TO PROFESSION/COMMUNITY

- Editorial board member for *Recent Patents on DNA And Gene Sequences*
- Reviewer for *Nucleic Acids Research, Expert Review of Molecular Diagnostics, Clinical Chemistry, Bioinformatics, Biotechniques*

TEACHING ACTIVITIES

- Lecturer, Molecular Medicine, CUHK-HKU-HKUST 2005-2006

TRAINEES:

Current

Trainee Name:	Ju Luan
Position:	Ph.D. student, The Chinese University of Hong Kong
Dates:	10/2005 – present
Project:	

Trainee Name:	Ling Yu
Position:	Ph.D. student, The Chinese University of Hong Kong
Dates:	01/2006 – present
Project:	

Trainee Name:	Xiaoxing Li
Position:	Ph.D.. student, The Chinese University of Hong Kong
Dates:	01/2007 – present
Project:	

Trainee Name:	Baohong Guo
Position:	Ph.D.. student, The Chinese University of Hong Kong
Dates:	starting 09/2007
Project:	

Previous

Trainee Name:	Ron McCullough
Position:	Ph.D. student, Boston University
Dates:	05/2004 – 01/2005 (current supervising on an informal basis)
Project:	Alternative splicing with MALDI-TOF MS for cancer diagnosis
Current Position:	Ph.D. student, Boston University

Trainee Name:	Shengnan Jin
Position:	Postdoc, Boston University
Dates:	10/2003 – 01/2005
Project:	Tissue-specific alternative first exon usage
Current Position:	Postdoc fellow, The Chinese University of Hong Kong

PUBLICATIONS

>510 Citations as of August, 2007.

Peer-Reviewed Publications

1. **Ding C** and Cantor CR, A high-throughput gene expression analysis technique using competitive PCR and MALDI-TOF mass spectrometry. ***Proc Natl Acad Sci U S A.*** 2003 Mar 18;100(6):3059-64, PMID: 12624187
2. **Ding C** and Cantor CR, Direct molecular haplotyping of long-range genomic DNA with multiplex PCR of single DNA molecules. ***Proc Natl Acad Sci U S A.*** 2003 Jun 24;100(13):7449-53, PMID: 12802015
3. Karaoz U, Murali TM, Letovsky S, Zheng Y, **Ding C**, Cantor CR, Kasif S, Whole genome annotation using evidence integration in functional linkage networks. ***Proc Natl Acad Sci U S A.*** 2004 Mar 2;101(9):2888-93, PMID: 14981259
4. Spira A, Beane J, Schembri F, Liu G, **Ding C,** Gilman S, Yang X, Cantor CR and Brody J, Noninvasive method for obtaining buccal mucosal RNA for measuring patterns of gene expression in epithelial cells from the mouth. ***Biotechniques.*** 2004 Mar;36(3):484-7, PMID: 15038164
5. **Ding C**, Maier E, Roscher AA, Braun A and Cantor CR, Simultaneous quantitative and allele-specific expression analysis with real competitive PCR. ***BMC Genet.*** 2004 May 5;5:8, PMID: 15128429
6. Isaacs FJ, Dwyer DJ, **Ding C**, Pervouchine DD, Cantor CR and Collins JJ, Highly specific riboswitches based on cis-repression and trans-activation. ***Nat Biotechnol.*** 2004 Jul;22(7):841-7, PMID: 15208640
7. **Ding C**, Chiu RW, Lau TK, Leung TN, Chan LC, Chan AY, Charoenkwan P, Ng IS, Law HY, Ma ES, Xu X, Wanapirak C, Sanguansermsri T, Liao C, Ai MA, Chui DH, Cantor CR, Lo YM, MS analysis of single nucleotide differences in circulating nucleic acids: application to noninvasive prenatal diagnosis. ***Proc Natl Acad Sci U S A.*** 2004 Jul 20;101(29):10762-7, PMID: 15247415
8. ENCODE Project Consortium, The ENCODE (ENCyclopedia Of DNA Elements) Project. ***Science.*** 2004 Oct 22;306(5696):636-40, PMID: 15499007

9. Rachlin R, **Ding C**, Cantor CR and Kasif S, MuPlex: Multi-Objective Multiplex PCR Assay Design, ***Nucleic Acids Res.*** 2005 Jul 1;33(Web Server issue):W544-7, PMID: 15980531
10. McCullough RM, Cantor CR and **Ding C**, High-throughput Alternative Splicing Quantification by Primer Extension and Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry. ***Nucleic Acids Res.*** 2005 Jun 20;33(11):e99, PMID: 15967806
11. Mao H, Ferguson TS, Cibulsky SM, Holmqvist M, **Ding C**, Fei H, Levitan IB, MONaKA, a novel modulator of the plasma membrane Na,K-ATPase. ***J Neurosci. 2005*** Aug 31;25(35):7934-43, PMID: 16135750
12. Rachlin R, **Ding C**, Cantor CR and Kasif S, Computational Tradeoffs in Multiplex PCR Assay Design for SNP Genotyping. ***BMC Genomics.*** 2005 Jul 25;6:102, PMID: 16042802
13. Chim SS, Tong YK, Chiu RW, Lau TK, Leung TN, Chan LY, Oudejans CB, **Ding C**, Lo YM., Detection of the placental epigenetic signature of the maspin gene in maternal plasma. (2005) ***Proc Natl Acad Sci U S A.*** 2005 Oct 11;102(41):14753-8, PMID: 16203989
14. Tsui NB, Chiu RW, **Ding C**, El-Sheikhah A, Leung TN, Lau TK, Nicolaides KH, Lo YM, Detection of Trisomy 21 by Quantitative Mass Spectrometric Analysis of Single Nucleotide Polymorphisms ***Clin Chem.*** 2005 Dec;51(12):2358-62, PMID: 16306096
15. Tong YK, **Ding C**, Chiu RW, Gerovassili A, Chim SS, Leung TY, Leung TN, Lau TK, Nicolaides KH, Lo YM, Noninvasive Prenatal Detection of Fetal Trisomy 18 by Epigenetic Allelic Ratio Analysis in Maternal Plasma: Theoretical and Empirical Considerations, ***Clin Chem.*** 2006 Oct 13; [Epub ahead of print] PMID: 17040955
16. Chan KC, **Ding C**, Gerovassili A, Yeung SW, Chiu RW, Leung TN, Lau TK, Chim SS, Chung GT, Nicolaides KH, Lo YM, Hypermethylated RASSF1A in Maternal Plasma: A Universal Fetal DNA Marker that Improves the Reliability of Noninvasive Prenatal Diagnosis, ***Clin Chem.*** 2006 Oct 26; [Epub ahead of print] PMID: 17068167
17. **Ding C**, Wong VW, Chow K, Chan HY, Hui AY, Wong GL, Lo YMD, Sung JJ, Chan HL, Quantitative Subtyping of Hepatitis B Virus Reveals Complex Dynamics of YMDD Motif Mutants Development during Long-Term Lamivudine Therapy, ***Antiviral Therapy*** 11:1041-1050
18. Lo YM, Tsui NB, Chiu RW, Lau TK, Leung TN, Heung MM, Gerovassili A, Jin Y, Nicolaides KH, Cantor CR, **Ding C.,** Plasma placental RNA allelic ratio permits noninvasive prenatal chromosomal aneuploidy detection, ***Nature Medicine*** 2007 Jan 7; PMID: 17206148
19. Chow KC, Chiu RW, Tsui NB, **Ding C**, Lau TK, Leung TN, Lo YM, Mass spectrometric detection of an SNP panel as an internal positive control for fetal DNA analysis in maternal plasma, ***Clin Chem.*** 2007 Jan;53(1):141-2.

Book Chapters and Reviews

20. **Ding C** and Cantor CR, Quantitative analysis of nucleic acids--the last few years of progress. ***J Biochem Mol Biol.*** 2004 Jan 31;37(1):1-10. PMID: 14761298.
21. **Ding C,** Qualitative and quantitative DNA and RNA analysis by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. ***Methods Mol Biol.*** 2006;336:59-71. PMID: 16916253
22. **Ding C.** and YM Lo, (2006) MALDI-TOF mass spectrometry for quantitative, specific and sensitive analysis of DNA and RNA. ***Ann N Y Acad Sci*** 2006 Sept; 1075:282-7. PMID: 17108222

23. **Ding C.** (2006) MALDI-TOF Mass Spectrometry for Analyzing Cell-free Fatal DNA in Maternal Plasma.. In "***Methods in Molecular Medicine: Prenatal Diagnosis***". Sinuhe Hahn and Laird G Jackson (editors). Humana Press. To appear.
24. **Ding C.** (2007) 'Other' applications of single nucleotide polymorphisms. ***Trends Biotechnol.*** 2007 May 8; [Epub ahead of print].

Patents

Eleven pending.

RECENT INVITED LECTURES

- A novel high throughput gene expression analysis technique using MALDI-TOF MS. The 3rd International Symposium on Current Technologies For Gene Expression Analysis, Nashville, TN, October 2002
- High-throughput gene expression analysis. Sequenom Inc., San Diego, CA, January 2003
- A novel high throughput DNA/RNA quantification technique. American Society for Biochemistry and Molecular Biology, San Diego, CA, April 2003.
- Non-invasive prenatal diagnosis of β-thalassemia by mass spectrometric analysis of fetal DNA in maternal plasma. American Society for Human Genetics, Los Angeles, CA, November 2003
- Fetal DNA in the maternal circulation. Boston University School of Medicine, Center for Human Genetics, Boston, MA, July 2004
- Nucleic acid analysis with MALDI-TOF mass spectrometry. 7th Annual Meeting of the Hong Kong Mass Spectrometry Society, Hong Kong, June 2005
- Automated MALDI-TOF Mass spectrometry as a powerful and versatile tool in molecular diagnostics. ACGA-Fudan 2005 International Symposium on Genomic Medicine, Shanghai, China, June 2005
- Mass spectrometry and its applications in analyzing cell free nucleic acids. Fourth International Conference on Circulating Nucleic Acids in Plasma/Serum, London, UK, September 2005
- Mass spectrometry as a new tool for analysing fetal nucleic acids in maternal plasma. 1st Asia Pacific Congress in Maternal Fetal Medicine, Hong Kong, China, October 2005
- Placental Transcript in maternal plasma: towards non-invasive fetal gene expression profiling. Transcriptome 2005, Shanghai, China, November 2005
- Non-invasive prenatal diagnosis. 10th International conference on Thalassaemia and Haemoglobinopathies, Dubai, UAE, January 2006.
- Qualitative and quantitative nucleic acid analysis with MALDI-TOF mass spectrometry. CANCER 2006, Hong Kong, China, April 2006.
- Noninvasive Prenatal Diagnosis by Analysis of Fetal DNA and RNA in Maternal plasma, ICGCG2006, Beijing, August 2006
- Circulating nucleic acids as biomarkers for molecular diagnosis, State key laboratory of Oncology in Southern China, Sun Yat-sen University, February 2007

FUNDING SUPPORT

Current support

Agency:	Research Fund for the Control of Infectious Diseases
Project:	Viral mutant discovery in HBV quasi-species in patients undergoing long-term lamivudine treatment
Role:	Principal Investigator
Dates:	2007/10/01 – 2009/9/31
Total Direct Cost:	HK$649,504

Agency:	NIH
Project:	The airway transcriptome as a biomarker for lung cancer
Role:	Co-Principal Investigator
Dates:	2005/10/1 – 2008/9/31
Total Direct Cost:	US$250,000
Percent Effort:	20% (Reduced to 0% due to relocation to Hong Kong in Feb, 2005)

Agency:	Hong Kong Research Grant Council
Project:	Polymorphisms in IKBa Gene and their Associations with Gastric Cancer
Role:	Co-Principal Investigator
Dates:	2006/07/30 – 2008/06/31
Total Direct Cost:	HK$654,500

Agency:	Hong Kong ITF fund
Project:	Development of an accurate blood test for the detection and monitoring of hepatocellular carcinoma
Role:	Co-Principal Investigator
Dates:	2006/09/01 – 2007/08/31
Total Direct Cost:	HK$862,000

Previous support

Agency:	NIH (1R01HG03110-01) P
Project:	Alternative promoter usage in tissue-specific gene expression
Role:	Co-Principal Investigator
Dates:	2003/10/1 – 2006/9/31
Total Direct Cost:	US$915,850
Percent Effort:	50% (Reduced to 0% due to relocation to Hong Kong in Feb, 2005)

Agency:	The Chinese University of Hong Kong,
Project:	Unknown viral mutant discovery and simultaneous quantification using base-specific cleavage and MALDI-TOF mass spectrometry
Role:	Principal Investigator
Dates:	2005/10/1 – 2006/9/31
Total Direct Cost:	HK$80,000

Agency:	The Chinese University of Hong Kong
Project:	Characteristics of circulating cell-free DNA in the plasma
Role:	Principal Investigator
Dates:	2007/06/30 – 2008/05/31
Total Direct Cost:	HK$41,000